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Effect of Prior Influenza Virus Infection on Susceptibility of AKR/J Mice and Squirrel Monkeys to Respiratory Challenge with *Legionella pneumophila*

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In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the author do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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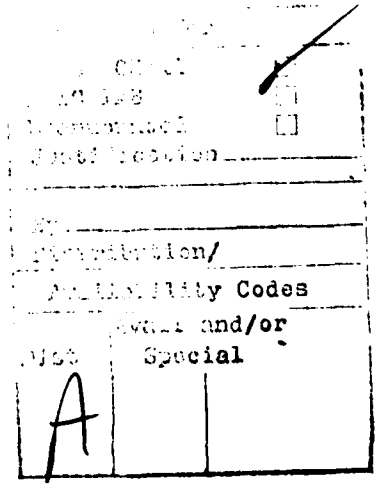

ABSTRACT

AKR/J mice and squirrel monkeys show greater mortality when exposed to a sequence of influenza virus and Legionella pneumophila than to either agent alone.

As knowledge of Legionnaires' disease has accumulated, the evidence suggests that many infections occur in individuals with underlying disease. Since Legionella pneumophila appears to spread by the airborne route (5, 6, 8, 9), we have initiated a study of respiratory virus infection as a predisposing factor for L. pneumophila infection in experimental animals.

The virus selected was a mouse-adapted variant of the Aichi/2/68 strain of type A influenza virus (H3N2). The virus was propagated in embryonated eggs (10). The virus titer in the allantoic fluid that was harvested was estimated to be $10^{8.2}$ median egg infectious doses (EID_{50})/ml.

The Philadelphia-1 strain of L. pneumophila was grown on charcoal-yeast extract (CYE) agar as previously described (3). Enumeration of bacteria was accomplished by spreading 0.1 ml of the appropriate dilution on CYE agar and counting colonies after incubation.

Two hosts were chosen for study: the AKR/J mouse, which has been reported to be susceptible to L. pneumophila challenge by Hedlund et al. (7), and the squirrel monkey, which demonstrates a mild clinical response to intratracheal instillation with either influenza virus (2) or L. pneumophila (unpublished observation).

For infection with both disease organisms mice were lightly anesthetized with halothane and inoculated intranasally (i.n.) with 0.05 ml of the appropriate dilution of organisms. Preliminary titrations with graded doses established the median lethal dose (LD_{50}) of influenza virus by this route to be $10^{4.8}$ EID_{50} and the LD_{50} of L. pneumophila, 1.1×10^{8} organisms. Fifteen mice in each of three groups were then treated as follows: one group received $10^{4.0}$ EID_{50} of influenza virus in 0.05 ml of heart-infusion broth (HIB); 3 days later, the mice were

given 0.05 ml of tryptose saline diluent. A second group was inoculated with virus and 3 days later were inoculated i.n. with 10^6 L. pneumophila. The third group received 0.05 ml of HIB followed 3 days later by 10^6 L. pneumophila. Results are shown in Table 1. The mortality rate among the mice given the sequence of virus followed by bacteria was significantly higher than that of either of the single organism control groups.

Confirmatory experiments with monkeys were restricted in scope because of cost and availability. The reaction of squirrel monkeys to influenza virus has been described previously (2). Preliminary observations on groups of four monkeys each given 10^6 L. pneumophila either by intratracheal (i.t.) instillation or aerosol are described in Figure 1. Four additional monkeys served as controls. The technique for i.t. instillation has been described elsewhere, as have the procedures for aerosol exposure and subsequent dosage calculation (1). No monkeys died or were even markedly ill after challenge by either route. Such clinical signs as dyspnea, coughing, sneezing, nasal crusting and lethargy were present at various times, but were inconsistent and not considered as reliable indicators of infection. All i.t. instilled monkeys showed significant leukocytosis, anorexia, weight loss and increased respiratory rate; responses were somewhat less marked in aerosol-exposed monkeys. Table 2, which presents the serum microagglutination titers carried out by the method of Farshy et al. (4), indicates that the serological response was greater in i.t. infected monkeys.

To determine the effect of sequential respiratory infection 8 monkeys were inoculated i.t. with 10^7 EID_{50} of influenza virus, and four with sterile HIB. Three days later four of the eight influenza-infected monkeys

and the four HIB-instilled monkeys were exposed to an aerosol dose of 10^7 L. pneumophila. The four remaining influenza-infected animals were reserved as controls with no further treatment. Two of the four sequentially infected monkeys died, one on day 5 and one on day 7. The lungs of both contained at least 10^7 L. pneumophila. Unfortunately, histopathologic examination was not carried out. None of the monkeys in this experiment, including those that died, had fever higher than 103°F. The values for the four parameters discussed previously are shown in Figure 2. In the study case of the sequentially infected monkeys, these data are subject to bias because only two monkeys survived. Although the small numbers preclude statistical analysis, it appears that the response of sequentially-infected monkeys was more severe.

The sequence of influenza followed by Legionnaires' disease may be relatively uncommon in nature because of the differing seasonal patterns of the two diseases. What these data suggest, however, is the possibility that respiratory viruses may enhance host susceptibility to subsequent L. pneumophila infection.

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TABLE 1. Response of AKR/J mice to intranasal instillation of $10^{3.0}$ EID_{50} of influenza virus followed by 10^5 Legionella pneumophila

Treatment	Geometric mean time to death (days)	Dead/Total	P
Influenza virus alone on day 0	8.4	5/14	0.025[a]
Influenza virus on day 0 + *L. pneumophila* on day 3	7.1	13/15	0.005[b]
L. pneumophila alone on day 3	NA	0/15	

[a]Virus control vs. sequentially-infected mice, chi^2 test with Yates correction.

[b]Bacterial control vs. sequentially-infected mice, chi^2 test with Yates correction.

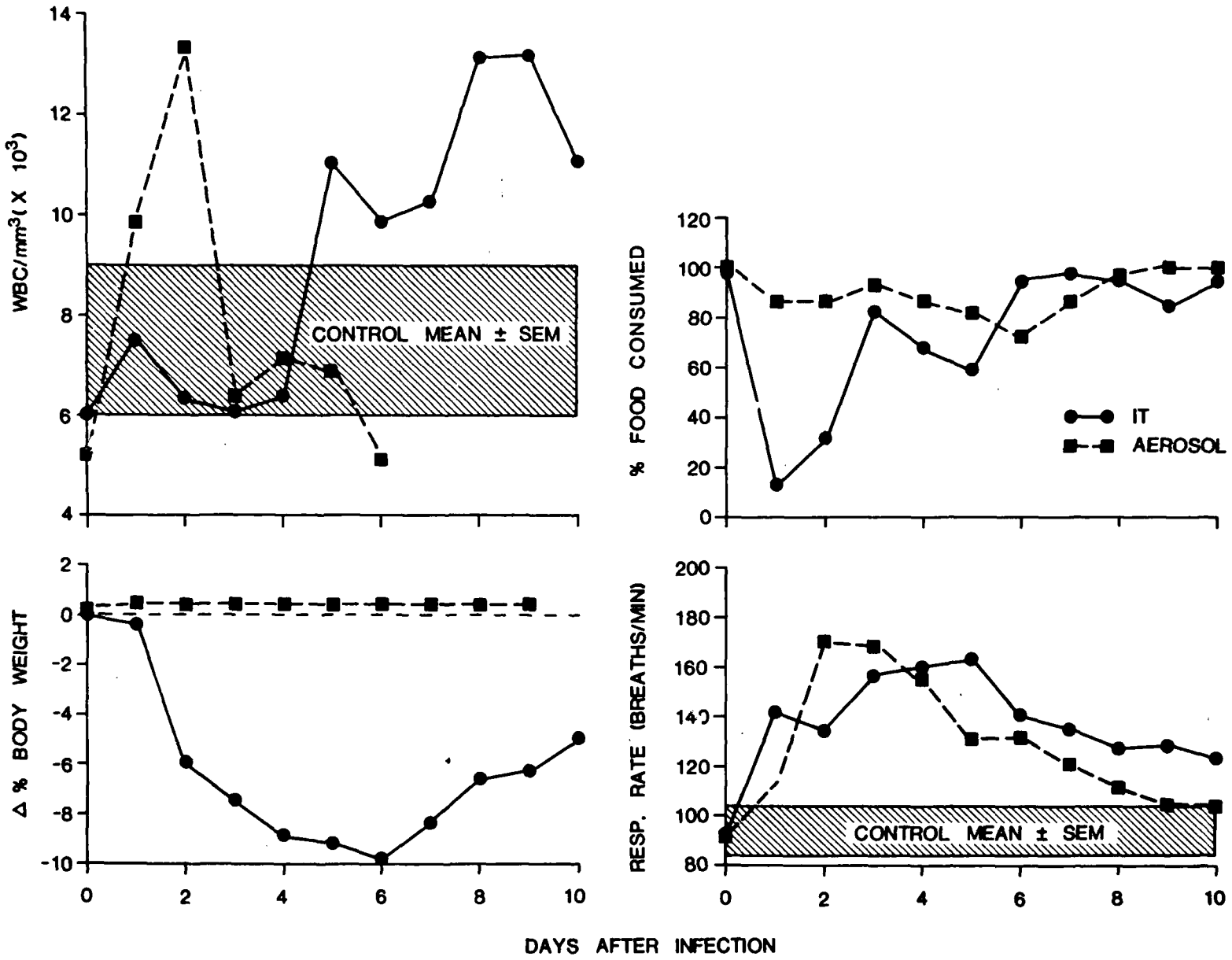
TABLE 2. Serum microagglutination (MA) titers in squirrel monkeys exposed to 10^6 Legionella pneumophila

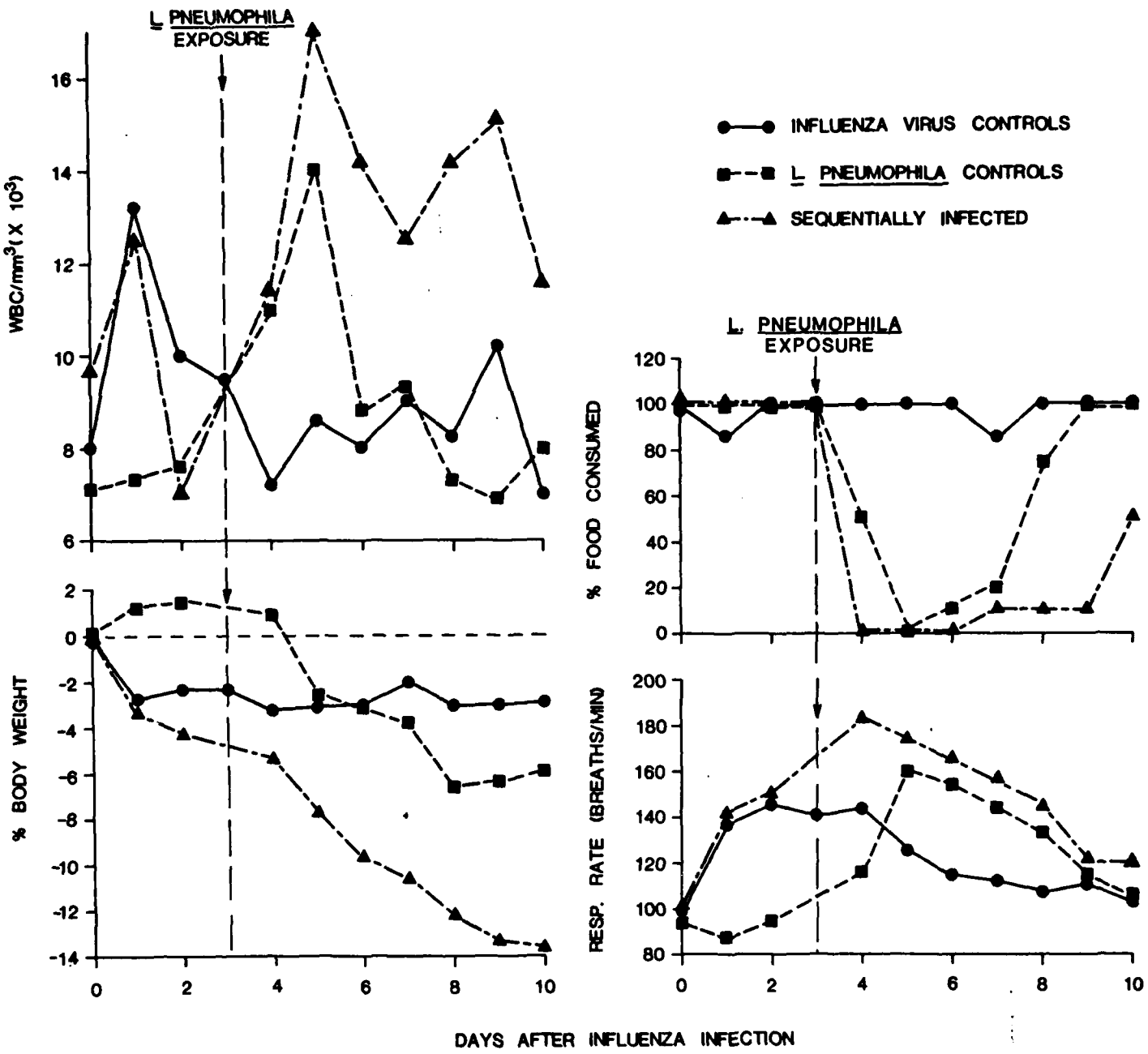
Days after infection	Geometric mean reciprocal MA titer	
	Intratracheal exposure (n = 6)	Aerosol exposure (n = 6)
0	< 8.0	< 8.0
14	64.0	32.0
28	256.0	45.3

FIGURE LEGENDS

FIG. 1. Response of squirrel monkeys to intratracheal or aerosol challenge with 10^6 Legionella pneumophila. Cross-hatched band is mean + SEM for uninfected controls. Control data are not shown for weight change (negligible) or food consumption (invariably 100%).

FIG. 2. Response of squirrel monkeys to sequential infection with influenza virus and L. pneumophila.


WBC/mm³ (X 10³)
CONTROL MEAN ± SEM
% FOOD CONSUMED
IT
AEROSOL
Δ % BODY WEIGHT
RESP. RATE (BREATHS/MIN)
CONTROL MEAN ± SEM
DAYS AFTER INFECTION


L. PNEUMOPHILA EXPOSURE
INFLUENZA VIRUS CONTROLS
L. PNEUMOPHILA CONTROLS
SEQUENTIALLY INFECTED
WBC/mm³ (X 10³)
% BODY WEIGHT
% FOOD CONSUMED
RESP. RATE (BREATHS/MIN)
DAYS AFTER INFLUENZA INFECTION